

Claims

1. An expression cassette comprising  
a polynucleotide sequence encoding a polypeptide including an HIV *Pol*  
5 polypeptide, wherein the polynucleotide sequence encoding said *Pol* polypeptide  
comprises a sequence having at least 90% sequence identity to the sequence presented of  
Figure 8 (SEQ ID NO:30); Figure 9 (SEQ ID NO:31) or Figure 10 (SEQ ID NO:32).
2. An expression cassette comprising  
10 a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:46, (ii) X equals Y, and (iii) Y is at least 97.
3. The expression cassette of claim 2, comprising  
15 a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:47, (ii) X equals Y, and (iii) Y is at least 144.
4. The expression cassette of claim 3, comprising  
20 a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:49 or SEQ ID NO:97, (ii) X equals Y, and (iii) Y is at least 300.
5. The expression cassette of claim 4, comprising  
25 a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:49, (ii) X equals Y, and (iii) Y is 2610.
6. The expression cassette of claim 4, comprising

a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:97, (ii) X equals Y, and (iii) Y is 2565.

5           7. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:51 (ii) X equals Y, and (iii) Y is 1494.

10           8. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:99, (ii) X equals Y, and (iii) Y is 1491.

15           9. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:55; SEQ ID NO:57; SEQ ID NO:101; SEQ ID NO:96; SEQ ID NO:134 or SEQ ID NO:135, (ii) X equals Y, and (iii) Y is at least 60.

20           10. The expression cassette of claim 9, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:55; SEQ ID NO:57; SEQ ID NO:101; SEQ ID NO:96; SEQ ID NO:134 or  
25   SEQ ID NO:135, (ii) X equals Y, and (iii) Y is 624.

11. An expression cassette comprising

a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:58; (ii) X equals Y, and (iii) Y is 354.

5           12. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:60; (ii) X equals Y, and (iii) Y is 876.

10           13. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:62; (ii) X equals Y, and (iii) Y is 3015.

15           14. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:103; (ii) X equals Y, and (iii) Y is 3009.

20           15. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:64 or SEQ ID NO:66; (ii) X equals Y, and (iii) Y is 297.

25           16. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of SEQ ID NO:68, (ii) X equals Y, and (iii) Y is 1965.

17. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:70; (ii) X equals Y, and (iii) Y is 1977.

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18. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:72 or SEQ ID NO:105, (ii) X equals Y, and (iii) Y is at least 30.

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19. The expression cassette of claim 18, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:72 or SEQ ID NO:105; (ii) X equals Y, and (iii) Y is 75.

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20. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:74 or SEQ ID NO:107, (ii) X equals Y, and (iii) Y is at least 30.

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21. The expression cassette of claim 20, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:74 or SEQ ID NO:107; (ii) X equals Y, and (iii) Y is 246.

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22. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:76; (ii) X equals Y, and (iii) Y is 1680.

23. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:78; (ii) X equals Y, and (iii) Y is 1668.

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24. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:80, SEQ ID NO:81 or SEQ ID NO:109; (ii) X equals Y, and (iii) Y is 216.

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25. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:83; (ii) X equals Y, and (iii) Y is 93.

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26. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:111; (ii) X equals Y, and (iii) Y is 90.

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27. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:85, or SEQ ID NO:113; (ii) X equals Y, and (iii) Y is 579.

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28. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:87; (ii) X equals Y, and (iii) Y is 288.

29. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:115; (ii) X equals Y, and (iii) Y is 287.

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30. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:89 or SEQ ID NO:117; (ii) X equals Y, and (iii) Y is at least 30.

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31. The expression cassette of claim 30 comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:89; (ii) X equals Y, and (iii) Y is 267.

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32. The expression cassette of claim 30 comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:117; (ii) X equals Y, and (iii) Y is 261.

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33. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:91; (ii) X equals Y, and (iii) Y is at least 30.

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34. The expression cassette of claim 33 comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:91; (ii) X equals Y, and (iii) Y is 321.

35. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:93 or SEQ ID NO:94; (ii) X equals Y, and (iii) Y is 309.

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36. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:96; (ii) X equals Y, and (iii) Y is at least 60.

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37. The expression cassette of claim 36 comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:96; (ii) X equals Y, and (iii) Y is 624.

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38. An expression cassette comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:119; SEQ ID NO:120; SEQ ID NO:121; SEQ ID NO:122; SEQ ID NO:123;  
SEQ ID NO:124; SEQ ID NO:125; SEQ ID NO:126; SEQ ID NO:127; SEQ ID NO:131;  
SEQ ID NO:132 or SEQ ID NO:133, (ii) X equals Y, and (iii) Y is at least 60.

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39. The expression cassette of claim 38, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:119; SEQ ID NO:120; SEQ ID NO:121; SEQ ID NO:122; SEQ ID NO:123;  
SEQ ID NO:124; SEQ ID NO:125; SEQ ID NO:126; SEQ ID NO:127; SEQ ID NO:131;  
SEQ ID NO:132 or SEQ ID NO:133, (ii) X equals Y, and (iii) Y is at least 300.

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40. The expression cassette of claim 39, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:123 or SEQ ID NO:124, (ii) X equals Y, and (iii) Y is 2433.

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41. The expression cassette of claim 39, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:122, (ii) X equals Y, and (iii) Y is 2301.

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42. The expression cassette of claim 39, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:125; (ii) X equals Y, and (iii) Y is 2517.

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43. The expression cassette of claim 39, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:126 or SEQ ID NO:127, (ii) X equals Y, and (iii) Y is 2520.

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44. The expression cassette of claim 39, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:119, (ii) X equals Y, and (iii) Y is 1377.

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45. The expression cassette of claim 39, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:120 or SEQ ID NO:121, (ii) X equals Y, and (iii) Y is 1839.



46. The expression cassette of claim 39, comprising  
a polynucleotide comprising X contiguous nucleotides, wherein (i) the X  
contiguous nucleotides have at least 90% percent identity to Y contiguous nucleotides of  
SEQ ID NO:132 or SEQ ID NO:133, (ii) X equals Y, and (iii) Y is 1890.

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47. A polynucleotide comprising the sequence depicted in SEQ ID NO:33 or  
fragments derived therefrom.

48. The polynucleotide of claim 47, wherein said fragments comprise coding  
sequence for the gene products selected from the group consisting of Gag, Pol, Vif, Vpr,  
Tat, Rev, Vpu, Env and Nef.

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49. The polynucleotide of claim 48, wherein the fragment comprises a Gag gene  
product.

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50. The polynucleotide of claim 48, wherein the fragment comprises an Env gene  
product.

51. The polynucleotide of claim 50, wherein the Env gene product is gp160,  
gp140 or gp120.

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52. A polynucleotide comprising the sequence depicted in SEQ ID NO:45 or  
fragments derived therefrom.

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53. The polynucleotide of claim 52, wherein said fragments comprise coding  
sequence for the gene products selected from the group consisting of Gag, Pol, Vif, Vpr,  
Tat, Rev, Vpu, Env and Nef.

54. The polynucleotide of claim 53, wherein the fragment comprises a Gag gene product.

5 55. The polynucleotide of claim 53, wherein the fragment comprises an Env gene product.

56. The polynucleotide of claim 55, wherein the Env gene product is gp160, gp140 or gp120.

10 57. A polynucleotide comprising the sequence depicted in SEQ ID NO:128 or fragments derived therefrom.

15 58. The polynucleotide of claim 57, wherein the fragments comprise coding sequence for Env gene products gp160, gp140 or gp120.

59. The expression cassette of claim 1, further comprising one or more nucleic acids encoding one or more viral polypeptides or antigens.

20 60. The expression cassette of claim 59, wherein the viral polypeptide or antigen is selected from the group consisting of Gag, Env, vif, vpr, tat, rev, vpu, nef and combinations thereof.

25 61. The expression cassette of claim 1, further comprising one or more nucleic acids encoding one or more cytokines.

62. A recombinant expression system for use in a selected host cell, comprising, an expression cassette of claim 1, and wherein said polynucleotide sequence further comprises control elements capable of driving expression in the selected host cell.

63. The recombinant expression system of claim 62, wherein said control elements are selected from the group consisting of a transcription promoter, a transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences.

64. The recombinant expression system of claim 62 wherein said transcription promoter is selected from the group consisting of CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein.

65. A cell comprising an expression cassette of claim 1, and wherein said polynucleotide sequence further comprises control elements compatible with expression in the selected cell.

66. The cell of claim 65, wherein the cell is selected from the group consisting of a mammalian cell, an insect cell, a bacterial cell, a yeast cell, a plant, an antigen presenting cell, a primary cell, an immortalized cell, and a tumor derived cell.

67. The cell of claim 66, wherein the cell is selected from the group consisting of BHK, VERO, HT1080, 293, RD, COS-7, and CHO cells.

68. The cell of claim 67, wherein said cell is a CHO cell.

69. The cell of claim 66, wherein the cell is either *Trichoplusia ni* (Tn5) or Sf9 insect cells.

70. The cell of claim 66, wherein the antigen presenting cell is a lymphoid cell selected from the group consisting of macrophage, monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof.

71. A composition for generating an immunological response, comprising an expression cassette of claim 1.

5 72. The composition of claim 71, further comprising one or more *Pol* polypeptides.

73. The composition of claim 72, further comprising an adjuvant.

10 74. A composition for generating an immunological response, comprising an expression cassette of claim 52.

75. The composition of claim 74, further comprising a *Pol* polypeptide.

15 76. The composition of claim 74, further comprising one or more polypeptides encoded by the nucleic acid molecules encoding a viral polypeptide or antigen selected from the group consisting of Gag, Env, vif, vpr, tat, rev, vpu, nef and combinations thereof.

20 77. The composition of claim 76, further comprising an adjuvant.

78. A method of immunization of a subject, comprising,  
introducing a composition of claim 71 into said subject under conditions that are compatible with expression of said expression cassette in said subject.

25 79. The method of claim 78, wherein said expression cassette is introduced using a gene delivery vector.

80. The method of claim 79, wherein the gene delivery vector is a non-viral vector.

81. The method of claim 79, wherein said gene delivery vector is a viral vector.

82. The method of claim 79, wherein said gene delivery vector is selected from the group consisting of an adenoviral vector, a vaccinia viral vector, an AAV vector, a retroviral vector, a lentiviral vector and an alphaviral vector.

83. The method of claim 82, wherein said gene delivery vector is a Sindbis-virus derived vector.

84. The method of claim 82, wherein said gene delivery vector is a cDNA vector.

85. The method of claim 82, wherein said gene delivery vector is a eukaryotic layered viral initiation system (ELVIS).

86. The method of claim 79, wherein said composition delivered using a particulate carrier.

87. The method of claim 79, wherein said composition is coated on a gold or tungsten particle and said coated particle is delivered to said subject using a gene gun.

88. The method of claim 79, wherein said composition is encapsulated in a liposome preparation.

89. The method of claim 79, wherein said subject is a mammal.

90. The method of claim 89, wherein said mammal is a human.

91. A method of generating an immune response in a subject, comprising: providing an expression cassette of claim 1,

expressing said polypeptide in a suitable host cell,  
isolating said polypeptide, and  
administering said polypeptide to the subject in an amount sufficient to elicit an  
immune response.

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92. A method of generating an immune response in a subject, comprising  
introducing into cells of said subject an expression cassette of claim 1, under  
conditions that permit the expression of said polynucleotide and production of said  
polypeptide, thereby eliciting an immunological response to said polypeptide.

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93. The method of claim 92, where the method further comprises co-  
administration of an HIV polypeptide.

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94. The method of claim 93, wherein co-administration of the polypeptide to the  
subject is carried out before introducing said expression cassette.

95. The method of claim 93, wherein co-administration of the polypeptide to the  
subject is carried out concurrently with introducing said expression cassette.

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96. The method of claim 93, wherein co-administration of the polypeptide to the  
subject is carried out after introducing said expression cassette.

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97. The expression cassette of claim 59, wherein the viral polypeptide or antigen  
is selected from the group consisting of polypeptides derived from hepatitis B, hepatitis C  
and combinations thereof.